

Randomized, double blind, prospective trial investigating the efficacy of Methotrexate in induction and maintenance of steroid free remission in ulcerative colitis (MEthotrexate Response In Treatment of UC - MERIT-UC)

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1 SUMMARY

Title:	Randomized, double blind, prospective trial investigating the efficacy of Methotrexate in induction and maintenance of steroid free remission in ulcerative colitis (MEthotrexate Response In Treatment of UC - Merit-UC)
Research Hypothesis:	MTX applied subcutaneously (sq) once weekly is effective to induce and maintain steroid free remission or response in patients with active ulcerative colitis, who have either failed 5-aminosalicylic acid (5-ASA) therapy, or are steroid dependent or have failed or are intolerant to azathioprine or 6-mercaptopurine therapy or failed to respond or lost response to anti-TNF therapy, compared to placebo.
Phase:	II
Study design:	Double-blind, placebo controlled, randomized, multicenter, two parallel groups
Study population:	Patients with active ulcerative colitis who are either failing 5-aminosalicylic acid (5-ASA) therapy, or are steroid dependent or have failed or are intolerant to azathioprine or 6-mercaptopurine therapy or failed to respond or lost response to anti-TNF therapy.
Sample size:	220 patients
Treatment:	<p>Induction period (week 1-16):</p> <ul style="list-style-type: none"> • 25 mg MTX sq once weekly + Steroid taper + 1 mg folic acid daily <p>Maintenance period (week 17-48) (Randomization):</p> <ul style="list-style-type: none"> • 25 mg MTX sq once weekly + 1 mg folic acid daily + 2.4 g mesalamine • Placebo sq once weekly + 1 mg folic acid daily + 2.4 g mesalamine
Treatment duration:	The study comprises an open label <i>Induction Period</i> of 16 weeks and a placebo controlled <i>Maintenance Period</i> of 32 weeks. In the <i>Induction Period</i> the patient will be treated with MTX 25 mg/once weekly + daily folic acid and a prednisone-tapering schedule for 10-12 weeks starting at either 40 mg or 20 mg depending on the need for steroids 2 weeks before inclusion in the study. Additionally, a reduction to MTX 15 mg once weekly is possible in case of MTX associated side effects.

	<p>If the patient has responded (as defined by a reduction from baseline of a partial Mayo score ≥ 2 points and at least 25% with an accompanying decrease in the rectal bleeding subscore of ≥ 1 point or an absolute rectal bleeding subscore of 0-1 point and has a clinical Mayo score ≤ 5 or is in remission (as defined as a partial Mayo score of 2 points or lower with no individual subscore exceeding 1 point) <u>and</u> is off steroids after 16 weeks of therapy with MTX 25 mg/weekly, the patient will be randomized to either MTX 25 mg/weekly¹ + 2.4 g 5-ASA + folic acid or placebo + 2.4 g 5-ASA + folic acid until week 48</p> <p>¹<i>in case of previous dose reduction in the induction phase the patient will be treated with 15 mg MTX weekly. Also a dose reduction in the Maintenance Period in case of suspected MTX associated side effects is possible.</i></p>
Main criteria for inclusion:	<ul style="list-style-type: none"> • Signed informed consent. • Man or woman between 18 and 70 years of age. • UC diagnosed by routine clinical, radiographic, endoscopic, and pathological criteria. • Active UC with a Mayo score of 6 to 12 points and moderate-to severe active disease on sigmoidoscopy (Mayo endoscopic subscore of at least 2) <u>and</u> at least <u>one</u> of the following criteria <ul style="list-style-type: none"> ○ Steroid dependent UC as defined by the Clinical Trials Task Force of the International Organization of Inflammatory Bowel Disease (IOIBD)² ○ Primary failure or loss of response to an anti-TNF therapy in the past ○ Primary failure or loss of response to vedolizumab in the past ○ Intolerance/failure of azathioprine/6-MP therapy in the past ○ Failure of 5-ASA therapy <p><i>(²Steroid dependence is defined as a clinical response to treatment with prednisone 40 to 60 mg/day and relapse within 30 days after prednisone treatment was completed or as a requirement for a daily dosage of not less than 10 mg of prednisone and impossibility of weaning the patient off steroid without clinical relapses {two attempts to discontinue the medication within the preceding six months of the start of the study}).</i></p>
Main criteria for exclusion:	<ul style="list-style-type: none"> • Failure to respond to 40 mg of prednisone or higher/day in the last 2 weeks before inclusion (Week 0 Visit) • Azathioprine (AZA) or 6-mercaptopurine (6-MP) must be discontinued at least 2 weeks before inclusion into the study (Week 0 Visit)

	<ul style="list-style-type: none"> • Anti-TNF therapy (Infliximab (Remicade), Adalimumab (Humira), Golimumab (Simponi)), in the last 4 weeks before the Week 0 Visit • Vedolizumab (Entyvio) in the last 4 weeks before the Week 0 Visit • Failure of cyclosporine therapy in the previous 6 months prior to Screening visit • Patients with serum albumin < 2.5 g/dl at baseline • Low serum folate defined as decrease of >10% below normal range • Patients with WBC < 3.0 x10⁹/L at baseline • Patients with platelet count < 100 x10⁹/L • Patients with initial elevation of AST or ALT > 1.5 times above normal limit at baseline • Patients with an underlying infection with C. difficile at Screening visit • Patients with pre-existing hepatic disease • Patients with known non-alcoholic fatty liver disease (NAFLD) • Patients with known Hepatitis B or Hepatitis C • Patients with pre-existing renal dysfunction (creatinine >1.5 mg/dl). • Patients with a pre-existing chronic lung disease other than well controlled asthma • Patients with interstitial lung disease of unknown cause • Patients with a BMI >35 • Known previous or concurrent malignancy (other than that considered surgically cured, with no evidence for recurrence for 5 years). A recent history of basal cell or squamous cell carcinoma, which is considered surgically cured, does not exclude the subject. • Existing pregnancy, lactation, or planned pregnancy* (men and women) within the next 12 months. (*Methotrexate should not be used for at least 3 months before planned pregnancy for men and women and should not be used during pregnancy or breast feeding) • High alcohol consumption (more than seven drinks per week) • Non - steroidal inflammatory medications (NSAIDs) as long-term treatment, defined as use for at least 4 days a week each month • Continuous treatment with one of the following drugs: <ul style="list-style-type: none"> ○ Probenecid,
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	<ul style="list-style-type: none"> ○ Trimethoprim/sulfamethoxazole ○ Sulfasalazine ○ Acitrecin, ○ Streptozocin <ul style="list-style-type: none"> • Non-use of appropriate contraceptives in females of childbearing potential (e.g. condoms, intrauterine device {IUD}, hormonal contraception, or other means considered adequate by the responsible investigator) or in males with a child-fathering potential (condoms, or other means considered adequate by the responsible investigator during treatment) • Participation in another clinical trial within the last 30 days, simultaneous participation in another clinical trial, or previous participation in this trial • Well-founded doubt about the patient's cooperation.
Concomitant medication:	<p>Following drug groups <u>are permitted</u> as concomitant medication:</p> <ul style="list-style-type: none"> • Orally administered mesalamine containing drugs (which should be kept at a stable dose throughout the trial) and should be at a stable dose 2 weeks before inclusion in the study. Every patient (except those with known intolerance of 5-ASA) will receive 2.4g mesalamine in the Maintenance Period. • Local therapies containing 5-ASA (enemas, suppositories) or steroid enemas should be at a stable dose 2 weeks before inclusion in the study. • Promethazine and/or ondansetron for treatment of MTX associated nausea <p>Following drug groups <u>are not permitted</u> as concomitant medication:</p> <ul style="list-style-type: none"> • Cytostatics/immunosuppressants, cyclosporine, tacrolimus, mycophenolate mofetil, 6-MP, azathioprine. • Anti-TNF therapies (Infliximab (Remicade), Adalimumab (Humira) Golimumab (Simponi)). • ,Vedolizumab (Entyvio) • Long-term oral antibiotics as defined as use for >14 days each month. • Trimethoprim/sulfamethoxazole • Probenecid

	<ul style="list-style-type: none"> • Sulfasalazine • Acitrecin • Streptozocin • Non - steroidal anti-inflammatories (NSAIDs) as long-term treatment, defined as use for at least 4 days a week each month • No live vaccines should be administered during the trial • Oral or intravenous corticosteroids with the exception of initial tapering
Criteria for Evaluation:	<p>The severity of the mucosal disease will be evaluated by two sigmoidoscopies (week 0 and week 48)</p> <p>The Mayo Score will be used as the disease activity index. The Mayo Scoring system ranges from 0 - 12 points and is a composite index consisting of 4 disease variables (stool frequency, rectal bleeding, endoscopy evaluation, and Physician's Global Assessment). A clinical response in this trial is defined as a reduction from baseline in the clinical Mayo score of ≥ 2 points and at least 25%, with an accompanying decrease in the rectal bleeding subscore of ≥ 1 point or an absolute rectal bleeding subscore of 0-1 point and a clinical Mayo score ≤ 5. Clinical remission is defined as a Mayo score of ≤ 2 points with no individual subscore exceeding 1 point.</p>
Primary variables	<ul style="list-style-type: none"> • Relapse-free survival, comprised of three components, all of which must be met to be categorized as relapse-free: total week 32 Mayo score not exceeding 2 points, with all individual subscores not exceeding 1 point and relapse free survival defined by a numerical stable Mayo score throughout 32 weeks of maintenance therapy without increase of 3 or more points in the partial Mayo clinic score (excluding sigmoidoscopy) compared to the partial Mayo score of the individual patient at randomization at week 16 (2) <u>and</u> no steroid use or other immunosuppressive medication (anti-TNF agents, thiopurines, cyclosporine, tacrolimus) to control disease activity throughout the 32 week maintenance period.
Secondary variables:	<ul style="list-style-type: none"> • Mucosal healing defined as an absolute subscore for endoscopy of 0 or 1 at week 48. • Relapse of disease in the <i>Maintenance period</i> as defined as an increase of 3 or more points in the partial Mayo clinic score (excluding sigmoidoscopy) with an absolute clinical Mayo score ≥ 4 <u>or</u> need for re-treatment with steroids.
Statistical analysis:	<p>The primary aim is to test effectiveness of MTX in maintaining clinical remission over 32 weeks of therapy compared to placebo. Analyses of this</p>

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	<p>aim will be conducted at two time points: (1) final analyses at the conclusion of the trial and (2) one interim analysis when about half of the patients completed follow-up in the maintenance phase. The interim analysis is planned to allow the DSMB to determine whether treatments are so convincingly different that continuation of the trial would be unethical, and also whether side effects of treatment are too severe to warrant continued therapy given the potential risk: benefit ratio.</p> <p>In addition, we plan to conduct a single analysis of open label induction phase remission after the first 75 patients have completed this phase of the trial. Accrual of sufficient numbers of patients into the randomized maintenance trial depends on the effectiveness of MTX in achieving remission during the 16 week open label induction phase. Therefore, we will test whether response and remission rates during induction in the first 75 patients are sufficient to meet goals for accrual to randomization in a timely manner.</p>
Duration of study	12 months of therapy per patient. The anticipated time of the overall study duration is 4 years
Number of centres:	25-30 centers in United States

2 INTRODUCTION AND BACKGROUND

Methotrexate (MTX), which was initially developed in 1948 for the treatment of leukemia, has been clinically used for nearly 60 years as low and high dose therapy in leukemias, certain lymphomas, Wegeners' disease, psoriasis and rheumatoid arthritis. In inflammatory bowel disease the clinical efficacy has been established for steroid dependent Crohn's disease (CD).

Ulcerative colitis (UC) is a chronic inflammatory gastrointestinal disorder, which is limited to the colon and characterized by the involvement of the mucosa only (in contrast to the transmural inflammation seen in CD). UC primarily affects young adults (20 - 40 years) but may present also at a very early age (5-10 years) or in later in life (>60 years). The inflammatory process in UC is primarily localized to the rectum (proctitis) or can extend proximally in a contiguous manner involving the mucosa up to the splenic flexure (left sided colitis) or involving the entire colon (extensive colitis). A key clinical feature of UC is bloody diarrhea. The clinical course of UC is characterized by periods of spontaneous exacerbation (acute flares) and remission (50 - 80% of patients) while some patients have a continuous active disease course (15 - 30% of patients) and others develop severe colitis (5 - 10% of patients), which can result in colectomy if medical therapy is not effective. Long standing UC is associated with an increased risk of colorectal cancer; the use of 5-ASA products may reduce this risk. UC can also be associated with inflammation involving extra-intestinal sites such as the skeletal, skin, or biliary system.

Clinical pharmacology of MTX

MTX is an analogue of folic acid and of aminopterin, which is also a folic acid antagonist. One of the main mechanisms of action is the inhibition of dihydrofolate reductase. This enzyme is involved in the de novo synthetic pathway for purines and pyrimidines. The rationale for the use of high dose MTX in the treatment of cancer is that the fast proliferating malignant cells become starved of purine and pyrimidine precursors and therefore are not able to sufficiently maintain DNA and RNA synthesis leading to a decreased proliferation. In contrast, the underlying anti-inflammatory effect of low-dose MTX in inflammatory diseases such as rheumatoid arthritis, psoriasis and inflammatory bowel diseases is less clear, since the anti-proliferative activity of low-dose MTX is minimal. Multiple mechanisms of actions are proposed including promotion of adenosine release, inhibition of production of pro-inflammatory cytokines, suppression of lymphocyte proliferation, neutrophil chemotaxis and adherence and reduction of serum immunoglobulin's ^{1,2}.

MTX can be administered by oral, subcutaneous, intramuscular or intravenous routes. Orally, MTX is absorbed in the jejunum and good bioavailability is only achieved in doses below 15 mg. Above this threshold absorption rates can vary up to 30% ³. The serum half-life of MTX is approximately 7-10 hours, but some patients have a prolonged serum half-life for up to 26 hours. After MTX is taken up by tissues, it is converted to methotrexate polyglutamates, which are long-lived derivatives that retain biochemical and biological activity. Sixty-five to eighty percent of the drug is excreted by the kidneys (the major part in

the first 12 hours after administration) and 20-35% is secreted with the bile. Plasma MTX measurements are clinically not meaningful in defining an optimal plasma concentration ⁴.

Adverse event profile and toxicity of MTX

The most often observed adverse event of MTX is nausea. Other significant adverse events include hepatotoxicity, bone marrow suppression ⁵ and rarely hypersensitivity pneumonitis ⁶. Due to the potential teratogenicity, MTX is contraindicated in pregnancy ⁷. Elevations of liver enzymes (AST, ALT, AP) is observed in up to 25% of the patients, but the development of hepatic fibrosis or cirrhosis probably due to the intrahepatic accumulation of MTX metabolites is overall a rare event ⁸. In the first trial by Feagan et al, investigating the efficacy of 25 mg once weekly sq MTX in the induction of a steroid free remission in patients with steroid dependent CD, 7 patients (7.4%) had to be withdrawn due to elevated LFT's, whereas no patient experienced elevations of LFT's warranting withdrawal from the trial in the maintenance study (15 mg MTX once weekly sq) ^{9, 10}. No significant toxicities of MTX 25 mg /weekly intramuscularly (im) applied over 50 weeks were reported in a recently completed study comparing the combination therapy of MTX and infliximab with infliximab alone in patients with CD ¹¹.

Effectiveness of MTX in inflammatory bowel diseases

The effectiveness of intramuscularly administered MTX 25 mg once weekly in steroid dependent CD is well documented. In the North American Crohn's Study Group landmark trial, 39.4% of the patients receiving MTX 25 mg sq/week were in clinical remission, as defined as a Crohn's disease activity index (CDAI) < 150 points and the discontinuation of prednisone, compared to 19.1% of the patients in the placebo group ¹⁰. Of note is that two smaller placebo controlled studies with orally administered lower doses of MTX did not demonstrate a statistical significant difference to placebo, suggesting that the route of administration and the dose of MTX play an important role ^{12, 13}. Feagan et al. also demonstrated a significant efficacy of 15 mg MTX sq once weekly in maintaining steroid free remission in CD patients (after 40 weeks 65% remission in the MTX group vs. 39% in the placebo group) ⁹.

So far no large randomized controlled trials have investigated the efficacy of MTX in patients with UC. Kozarek et al. reported the successful induction of remission in five of seven patients with UC with MTX 25 mg given once weekly im ¹⁴. Baron et al. also observed a steroid sparing effect of MTX 15 mg orally once weekly in a small group of UC patients ¹⁵. Two small, randomized, placebo controlled trials investigated the efficacy of oral MTX in steroid dependent patients and did not find a significant therapeutic efficacy compared to placebo ^{16, 17}. However, MTX was administered orally at relative low doses (12.5 or 15 mg once weekly). A clear dose response has been observed in patients with rheumatoid arthritis ¹⁸, suggesting that higher doses of MTX administered either sq or im could be clinically effective in UC. Paoluzi et al. compared azathioprine (2 mg/kg body weight/day) to MTX (12.5 mg/kg im once weekly) in patients with steroid dependent or

refractory UC ¹⁹. In this prospective single center open label study 69% (22/32) and 19% (6/32) of the patients on AZA went into remission or experienced an improvement of symptoms, respectively, compared to 60% (6/10) and 40% (4/10) in the MTX group. Additionally, several single center observations published in the last 2-3 years employing higher doses of MTX suggest a high clinical efficacy of MTX in purine analog (azathioprine, 6-mercaptopurine) intolerant UC patients or in UC patients with steroid dependent disease course. Cummings et al. analyzed retrospectively the efficacy of orally administered MTX in 42 patients with either lack of benefit (n=11) or intolerance (n=31) to azathioprine and 8 patients with rheumatoid arthritis and UC with a primary MTX therapy for the rheumatoid arthritis ²⁰. The group from Oxford describes response and remission rates of 58% and 45% in azathioprine intolerant patients, 27% and 0% in previous azathioprine failure patients and 80% and 62% in patients with a concurrent rheumatoid arthritis. The mean dose for MTX was in the range of 20-25 mg. Nathan et al. report in a retrospective analysis of patients treated in a single hospital in Victoria, Australia, remission rates of 48% (11 of 23 patients) after subcutaneous administration of 20-25 mg MTX once weekly ²¹. Wahed et al. describe a clinical response, defined as steroid withdrawal, normalization of previously raised C-reactive protein or physician's clinical assessment of improvement) in 22/32 (68%) of UC patients ²² in a retrospective analysis of single University hospital in London. The analyzed group consisted mainly of patients, who were either previously intolerant (efficacy 14/21; 63%) or failed (efficacy 7/9; 78%) purine analogues.

JUSTIFICATION AND OBJECTIVES OF THE CLINICAL TRIAL

The therapeutic armamentarium for patients with active UC despite 5-aminosalicylates and steroids is considerably smaller than for patients with CD (therapeutic choices for steroid dependent UC patients: azathioprine, infliximab - therapeutic choices for steroid dependent CD patients: azathioprine, methotrexate, infliximab, adalimumab, certolizumab, natalizumab), there is clearly a need for further exploration of new therapies with a calculable risk profile for this patient group. Currently several new therapeutic approaches investigating the role of different "biologics" e.g. in the inhibition of the co-stimulation of T cells (Abatacept) or selective blockade of interactions between leukocytes and vascular endothelium (MLN 0002 antibody) are being tested in clinical trials. However, given the high costs of biologics, there is an additional need for affordable, easy to administer therapies such as MTX. Additionally, use of small molecules is favorable over biologics as the risk of anti-medication antibody formation is less likely. Since MTX is an old and non-patented drug, it is highly unlikely that this drug will be ever prospectively investigated in patients with UC.

Given the lack of large placebo controlled prospective studies investigating doses of MTX, which were clinically effective in inducing steroid free remission in CD patients, so far neither a negative nor a positive conclusion can be drawn from the published data about the clinical efficacy of MTX in UC. Due to an obvious dose-response relationship observed for

MTX in the treatment of rheumatoid arthritis, there seems to be an unmet need for investigating prospectively MTX in higher doses in patients with UC using a subcutaneous or intramuscular application.

3 PATIENT SELECTION

Inclusion criteria

- Signed informed consent.
- Man or woman between 18 and 70 years of age.
- UC diagnosed by routine clinical, radiographic, endoscopic, and pathological criteria.
 - Active UC with a Mayo score of 6 to 12 points and moderate-to severe active disease on sigmoidoscopy (Mayo endoscopic subscore of at least 2) and at least one of the following criteria
 - Steroid dependent UC as defined by the Clinical Trials Task Force of the International Organization of Inflammatory Bowel Disease (IOIBD) ²
 - Primary failure or loss of response to an anti-TNF therapy in the past
 - Primary failure or loss of response to vedolizumab in the past
 - Intolerance/failure of azathioprine/6-MP therapy in the past
 - Failure of 5-ASA therapy

²*Steroid dependence is defined as a clinical response to treatment with prednisone 40 to 60 mg/day and relapse within 30 days after prednisone treatment was completed or as a requirement for a daily dosage of not less than 10 mg of prednisone and impossibility of weaning the patient off steroid without clinical relapses {two attempts to discontinue the medication within the preceding six months of the start of the study}}.*

Exclusion criteria

Patients who meet one of the following criteria are to be excluded from the trial:

- Failure to respond to 40 mg of prednisone or higher/day in the last 2 weeks before inclusion (Week 0 Visit)
- Concomitant use of AZA or 6-MP (AZA or 6-MP therapy has to be stopped at least 2 weeks before inclusion into the study) (Week 0 Visit)
- Anti-TNF therapy (Infliximab (Remicade), Adalimumab (Humira) Golimumab (Simponi)) in the last 4 weeks before inclusion in the study (Week 0 Visit)
- Concomitant use of vedolizumab (Entyvio) in the last 4 weeks before the Week 0 Visit
- Failure of cyclosporine therapy in the previous 6 months prior to Screening visit
- Patients with serum albumin < 2.5 g/dl at baseline

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- Low serum folate defined as decrease of >10% below normal range
- Patients with $WBC < 3.0 \times 10^9/L$ at baseline
- Patients with platelet count $< 100 \times 10^9/L$
- Patients with initial elevation of AST or ALT > 1.5 times above normal limit at baseline
- Patients with an underlying infection with *C. difficile* at Screening visit
- Patients with pre-existing hepatic disease
- Patients with known non-alcoholic fatty liver disease (NAFLD)
- Patients with known Hepatitis B or Hepatitis C
- Patients with pre-existing renal dysfunction (creatinine >1.5 mg/dl)
- Patients with a pre-existing chronic lung disease other than well controlled asthma
- Patients with interstitial lung disease of unknown cause
- Patients with a BMI >35
- Known previous or concurrent malignancy (other than that considered surgically cured, with no evidence for recurrence for 5 years). A recent history of basal cell or squamous cell carcinoma does not exclude the subject.
- Existing pregnancy, lactation, or planned pregnancy* (men and women) within the next 12 months
(*Methotrexate should not be used for at least 3 months before planned pregnancy for men and women and should not be used during pregnancy or breast feeding)
- High alcohol consumption (more than seven drinks per week)
- Non-use of appropriate contraceptives in females of childbearing potential (e.g. condoms, intrauterine device {IUD}, hormonal contraception, or other means considered adequate by the responsible investigator) or in males with a child-fathering potential (condoms, or other means considered adequate by the responsible investigator during treatment)
- Non - steroidal anti-inflammatory medications (NSAIDs) as long-term treatment, defined as use for at least 4 days a week each month
- Continuous treatment with one of the following drugs:
 - Probenecid
 - Trimethoprim/sulfamethoxazole
 - Sulfasalazine
 - Acitrecin
 - Streptozocin

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- Participation in another clinical trial within the last 30 days, simultaneous participation in another clinical trial, or previous participation in this trial
- Well-founded doubt about the patient's cooperation

Concomitant medication

Following drug groups are permitted as concomitant medication:

- Orally administered mesalazine containing drugs (which should be kept at a stable dose throughout the trial) and should be at a stable dose 2 weeks before inclusion in the study. In the Maintenance Period, every patient (except those with known intolerance to 5-ASA) will receive 2.4 g mesalamine and no additional mesalamine should be administered.
- Local therapies containing 5-ASA (enemas, suppositories) or steroid enemas should be at a stable dose 2 weeks before inclusion in the study
- Promethazine/Ondansetron for treatment of MTX associated nausea

Following drug groups are not permitted as concomitant medication:

- Oral or intravenous corticosteroids with the exception of initial tapering
- Cytostatics/immunosuppressants, cyclosporine, tacrolimus, mycophenolate mofetil, 6-MP, azathioprine
- Anti-TNF therapies ((Infliximab (Remicade), Adalimumab (Humira) Golimumab (Simponi))
- Vedolizumab (Entyvio) Trimethoprim/sulfamethoxazole
- Probenecid
- Sulfasalazine
- Acitrecin
- Streptozocin
- Long-term oral antibiotics as defined as use for >14 days each month.
- Non - steroidal anti-inflammatories (NSAIDs) as long-term treatment, defined as use for at least 4 days a week each month
- No live vaccines should be administered during the trial

Overview of time sequence

The trial will begin with the **screening visit (days -14 to 0)**. The investigator will ask patients who are interested in participating in the study after a careful explanation, to sign an informed consent form prior to study specific examinations. The patient will receive a copy of the consent

form, which outlines the sequence of events in brief and summarizes the advantages and disadvantages of participation in the study.

After the patient has given his/her written consent, the screening examination will be performed to evaluate whether the patient is probably eligible for the study.

At the screening visit, a complete history will be taken including concomitant medication and the cumulative steroid dose of the last week prior to the screening visit and the demographic data will be collected. A physical examination will be performed and vital signs will be documented. The blood samples (max. 30 ml) will be taken from the patient (for hematology, serum chemistry, screening for hepatitis B and hepatitis C, beta-HCG) as well as the samples for the DNA, plasma and serum bank. Female patients with childbearing potential are only eligible for the study in case of a negative serum pregnancy test result. The patient has to provide a stool sample to exclude for *Clostridium difficile* infection. Also the patient will be provided with a stool collection kit to provide a stool sample for calprotectin determination, which should be brought back by the patient at the visit at week 0. A chest x-ray will be performed to exclude interstitial lung disease. Inclusion and exclusion criteria will be checked and completed.

Once the screening visit of the patient is entered in the database, a screening number will be assigned and the patient will receive 1 vial of MTX 2 ml (25mg/ml), 7 tablets folic acid 1 mg, and 2 tablets promethazine (25mg) by mail delivered to his home address from the investigational drug service. The patient should bring this vial and the folic acid tablets to the next visit at week 0. Depending on the local regulatory requirements the drug may be as well delivered to the investigational site.

The patient will also be provided with a diary, which he/she will be asked to complete every day in the evening for the last 3 days prior the next visit (week 0). At week 0 visit a sigmoidoscopy should be performed, to evaluate the degree of mucosal inflammation. The sigmoidoscopy score should be ≥ 2 , defined as moderate to severe active disease. Thereafter the full Mayo score should be calculated.

If the patient still fulfils all inclusion criteria and does not have an exclusion criterion the investigators will log onto the UNC data management center web site, and complete eligibility and exclusion criteria forms online. If the patient meets the criteria they will be assigned a unique study ID number that will appear on all future study instruments and medication packs.

The sigmoidoscopy at the week 0 visit may be omitted if the following requirements are met: (1) A colonoscopy was performed by a MERIT-UC investigator or sub-investigator within 14 days before the Screening visit of MERIT-UC. In this context the Week 0 Visit must be completed within 21 days of the colonoscopy. (2) The colonoscopy report must include the grade of inflammation in the recto-sigmoid colon so the Mayo score can be calculated. (3) Images of the recto-sigmoid colon should be obtained during the colonoscopy, if possible.

The patients will be then allocated to the methotrexate Induction Period:

25 mg MTX sq once weekly + steroid taper + 1 mg folic acid orally daily

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The patient will be instructed on proper injection technique, storage and disposal requirements prior to receiving first injection. The first injection of MTX will be administered at the investigational site at week 0, using the single MTX vial (2 ml, 25 mg/ml), which was delivered to the patient after the screening visit (or to the investigational site depending on local regulatory requirements). Once the patient received the first MTX injection at the investigational site and he should also start to take folic acid tablets 1 tablet (1 mg) daily. Each site will be supplied with syringes and sharp disposal containers. Patients with nausea after injection of MTX should be advised to take promethazine on the day of MTX injection.

After week 0 kits containing MTX vials for future injections and folic acid tablets will be delivered to the patient's home address or to the investigational site depending on the local regulatory requirements.

Every enrolled patient will be treated with systemic corticosteroids for 10 to 12 weeks according to a taper schedule outlined below.

Assignment to either the high or low dose prednisone taper will be made by the local investigator, based on the subject's individual treatment needs. Generally, patients will be grouped according to their previous steroid exposure. Patients who were receiving 20 mg or more of prednisone daily two weeks before randomization will be allocated to a higher steroid taper schedule (high dose prednisone stratum), the others to a lower steroid taper schedule (low-prednisone stratum). Depending on the subject's response, the local investigator may alter the rate of the taper during the induction phase. All subjects, however, must be off of steroids by the Week 12 Visit.

The steroid taper will follow the sequence described below.

Prednisone taper		
Stratum	High prednisone	Low prednisone
Screening	≤ 40 mg, > 20 mg	≤ 20 mg
Week 0	40 mg	20 mg
Week 2	30 mg	17.5 mg
Week 3	25 mg	15 mg
Week 4	20 mg	12.5 mg
Week 5	17.5 mg	10 mg
Week 6	15 mg	10 mg*
Week 7	12.5 mg	7.5 mg
Week 8	10 mg	5 mg
Week 9	7.5 mg	2.5 mg

Methotrexate response in treatment of UC

Week 10	5 mg	0 mg
Week 11	2.5 mg	
Week 12	0	
Table 1: Prednisone taper		
*Tapering is slowed due to the delayed onset of MTX activity, which is expected between week 6-8		

Induction Period

The Induction Period lasts for up to 16 weeks and includes **2 regular follow-up visits** (visits week 4, 12), **2 phone visits and blood draws** (visits week 2, 8) **and a visit at week 16**. Study visits during the Induction Period have a +/- 3 day window. The MTX dose of 25 mg once weekly can be reduced to 15 mg MTX in case of non-tolerable side effects such as nausea, which cannot be treated with promethazine or ondansetron, at the discretion of the local investigators. Promethazine tablets (25 mg to be taken on the day of MTX injection) will be provided by the investigational pharmacy directly to the patient, if concurrent nausea after the injection of MTX is reported by the local investigator. If promethazine is not effective in treating MTX induced nausea, ondansetron 4 mg tablets can be ordered by the investigator under certain precautions (see also “Medication preparation and distribution”). In case of elevations of liver enzymes or a drop of WBC due to MTX therapy there are special instructions in “Abnormal laboratory results”).

Maintenance Period

If patient responded (as defined by a reduction of the partial Mayo score ≥ 2 points and at least 25% with an accompanying decrease in the rectal bleeding subscore of ≥ 1 point or an absolute rectal bleeding subscore of 0-1 point and has a clinical Mayo score of ≤ 5) or is in remission (as defined as a partial Mayo score of ≤ 2 points with no individual subscore exceeding 1 point) and is off steroids after 16 weeks of therapy with MTX 25 mg/weekly, the patient will be randomized to the following therapy groups:

Group 1 Maintenance: 25 mg MTX* sq once weekly + 1 mg folic acid orally daily + 2.4 g 5-ASA

Group 2 Maintenance: Placebo sq once weekly + 1 mg folic acid orally daily+ 2.4 g 5-ASA

*In case of previous dose reduction the patient will be treated with 15 mg MTX/once weekly

The assigned drug regimen will be send directly to the patient or to the participating center, depending on local regulatory requirements. The study coordinator will call the subject to confirm that the medications were received. Documentation will be kept in the subject's study file.

If the patient failed to respond or still has a partial Mayo score ≥ 6 or needed steroid therapy after week 12, the patient cannot be randomized and therefore will be excluded from entering the Maintenance Period of the study. For these patients, the week 16 visit will be considered an early termination visit.

The Maintenance Period lasts 32 ± 1 week and includes **3 regular follow-up visits (visits week 24, 32, 40), 4 phone visits and blood draws (week 20, 28, 36, 44) and the final visit at week 48 which includes a sigmoidoscopy.** Study visits during the Maintenance Period have a ± 7 day window. Again the MTX dose of 25 mg once weekly can be reduced to 15 mg MTX in case of non-tolerable side effects such as nausea, which cannot be treated with promethazine or ondansetron, at the discretion of the local investigators. Promethazine 25 mg tablets or, if necessary, ondansetron 4 mg tablets (1 tablet to be taken on the day of MTX injection) will be provided by the investigational pharmacy directly to the patient, if concurrent nausea after the injection of MTX or placebo is reported by the local investigator (see also “Medication preparation and distribution”). In case of elevations of liver enzymes or a drop of WBC due to MTX therapy there are special instructions in “Abnormal laboratory results”). The mesalamine medications will be provided by the investigational pharmacy directly to the patient (see medication preparation and distribution). Patients not tolerating mesalamine should stop the drug, but will be continued in the study.

At each regular follow-up visit in the Induction Period and in the Maintenance Period (week 4, 12, 16, 24, 32, 40) a partial Mayo Score will be calculated, the concomitant medication (CM) and adverse events (AEs) will be assessed. The visit at week 48 will include a sigmoidoscopy for the calculation of the endoscopic Mayo score. Additionally at visit 2, 4, 8, 12, 16, 24, 32, 40 and 48 clinical laboratory examinations (hematology, serum chemistry) and at visit 4, 8, 12, 16, 24, 32, 40 and 48 urine samples for pregnancy tests will be performed and the vital signs will be measured (see timeline of study visit in appendix). Patients should bring stool samples for calprotectin evaluation at the screening visit and at week 16.

At each visit in week 4, 12, 16, 24, 32, 40 and 48 the partially used vials of the medication will be collected. The investigator shall compare whether the amount of actually administered vials concurs with the days in the study, and will enter his/her estimation concerning the patient’s compliance and the number of returned vials in the electronic CRF.

4 DESCRIPTION OF THE CLINICAL TRIAL SEQUENCE

Screening visit

Data to be recorded:

General parameters:

- Initials

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- Date of birth
- Sex
- Smoker (yes/no/ex-smoker)
- Race (NIH categories)
- Ethnicity (NIH categories)

Case history

- Date of confirmation of the diagnosis
- Classification of UC (proctitis, left sided colitis, pancolitis)
- Extraintestinal symptoms: type, duration of present symptoms
- Treatment of UC within the last year
 - type of medication
- Treatment of UC within the last 4 weeks
 - type of medication, dose

Examinations

- Vital signs: blood pressure (mm Hg), heart rate (min^{-1}), weight (kg), calculation of BMI
 - Height (cm)
 - Physical examination:
 - Head (including ENT and eyes)
 - Lymphatic system
 - Endocrine system
 - Lungs and respiratory tract
 - Peripheral vascular system
 - Cardiovascular system
 - Gastrointestinal tract, liver, spleen
 - Urogenital system
 - Nervous system
 - Muscular and skeletal system
 - Skin and connective tissue
 - Other
- Each: actual status: Present finding / normal condition / not examined
- Blood draw DNA, serum and plasma bank (to be sent to the central laboratory). Vials are provided by the central laboratory. These samples should be sent on dry ice to the central laboratory on the same day.
 - Blood sampling and measurement of laboratory parameters
 - Hematology

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- Serum chemistry + serum folate
- Screening for hepatitis B and C (HBsAg, anti-HBs, anti-HBc, Hep-C AB)
- Serum pregnancy test (only female patients with childbearing potential)
- - Stool sample for C. diff. toxin and calprotectin measurement
- Chest x-ray (if a chest x-ray was obtained within 1 year before screening, there is no need for a new chest x-ray, but the results and the date of this x-ray should be recorded in the CRF)

Formal aspects:

Informed consent procedure

Verification of inclusion / exclusion criteria (eligibility check), as far as possible

At the end of this visit all data should be immediately entered in the database, so that the Investigational Drug Service can send 1 vial MTX (2 mg/ml), 7 tablets folic acid, and 2 tablets of promethazine to the patient (or to the investigational site depending on local regulatory requirements) before the visit at week 0. The patient should bring the vial and the tablets to the visit at week 0.

Induction Period Visit week 0

- Vital signs: blood pressure (mm Hg), heart rate (min^{-1}), weight (kg)
- Height (cm)
- Physical examination:
 - Head (including ENT and eyes)
 - Lymphatic system
 - Endocrine system
 - Lungs and respiratory tract
 - Peripheral vascular system
 - Cardiovascular system
 - Gastrointestinal tract, liver, spleen
 - Urogenital system
 - Nervous system
 - Muscular and skeletal system
 - Skin and connective tissue
 - Other

Each: actual status: Present finding / normal condition / not examined

- Collect venous blood sample for hematology and serum chemistry for laboratory analyses (only if conducted > 14 days after screening)
- Sigmoidoscopy
- Training of injection technique with sodium chloride and injection of first MTX dose. Patient should start taking folic acid 1 tablet (1 mg) once daily.

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- The patient should be advised to take 1 25 mg tablet promethazine in case of nausea in the next 24-48 hours after the injection. In case of nausea the patient should also be advised to call the local coordinator for further supply of promethazine, which could be ordered by weborder from the investigational drug services (IDS). The IDS will then supply the patient with one promethazine tablet for each weekly MTX injection. In case promethazine is not effective, ondansetron can be directly ordered from the IDS (see “Medication and Distribution”)
- The supply of further drug and the injection utensils (see “Medication preparation and distribution”) will be shipped to the patient or handed to the patient at the investigational site depending on local regulatory requirements.

Formal Aspects:

Calculation of the Mayo score including sigmoidoscopy

Verification of inclusion / exclusion criteria (eligibility check)

Follow-up visits Induction Period = Visits Week 2, 8

- Measurement of laboratory parameters
 - Hematology
 - Serum chemistry
 - Urine pregnancy test (week 8 only)
- Phone interview with patient about adverse events (interview can be as well conducted in study center if feasible)

Follow-up visits Induction Period = Visits Week 4, 12

Examinations

- Vital signs: blood pressure (mm Hg), heart rate (min-1), weight (kg)
- Physical examination:
 - Head (including ENT and eyes)
 - Lymphatic system
 - Endocrine system
 - Lungs and respiratory tract
 - Peripheral vascular system
 - Cardiovascular system
 - Gastrointestinal tract, liver, spleen
 - Urogenital system
 - Nervous system
 - Muscular and skeletal system
 - Skin and connective tissue
 - Other

Each: actual status: Present finding / normal condition / not examined

Methotrexate response in treatment of UC

- Measurement of laboratory parameters
 - Hematology
 - Serum chemistry
 - Urine pregnancy test

Formal aspects:

- Return of study medication (empty vials)
- Calculation of Mayo score excluding the sigmoidoscopy score
- Compliance assessment (count of empty vials returned)
- Concomitant medication
- Adverse events
- Evaluate the progress of the steroid taper; patients should be off steroids by latest in week 12.
- Appointment for next visit
- At week 12 remind the patient to bring stool sample for next visit (container provided by central laboratory).

Final Visit Induction Period = Week 16 / premature withdrawal Induction Period

Examinations

- Physical examination:
 - Head (including ENT and eyes)
 - Lymphatic system
 - Endocrine system
 - Lungs and respiratory tract
 - Peripheral vascular system
 - Cardiovascular system
 - Gastrointestinal tract, liver, spleen
 - Urogenital system
 - Nervous system
 - Muscular and skeletal system
 - Skin and connective tissue
 - Other

Each: actual status: Present finding / normal condition / not examined

- Vital signs: blood pressure (mm Hg), heart rate (min-1), weight (kg)
- Measurement of laboratory parameters
 - Hematology

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- Serum chemistry
- Urine pregnancy test
- Stool sample for calprotectin stool levels.
- Plasma and serum collection for study repository

Formal aspects:

- Return of study medication (empty syringes)
- Compliance assessment (count of empty vials returned)
- Concomitant medication
- Adverse events
- Calculation of the partial Mayo score and evaluation if patient meets criteria for response or remission.
- **If patient meets criteria for response or remission, perform randomization for Maintenance Period. If the patient failed to respond or still needed steroid therapy after week 12, the patient cannot be randomized and must be excluded from the trial. In this case week 16 is the last study visit for the patient.**

In case of **premature withdrawal** from the study (yes, no)

If yes, reason:

- Intolerance of medication
- Lack of efficacy (need for steroids e.g. increase of steroids or re-introduction of steroids after previously successful taper)
- Severe adverse event
- Lack of patient's cooperation
- Inclusion criterion not fulfilled or exclusion criterion fulfilled coming to knowledge after recruitment
- Need for steroid therapy after week 12
- Other reason

Follow Up after premature withdrawal

If a subject withdraws before the completion of the study, follow up data will be collected one and three months after withdrawal. Information about which treatment options were started after withdrawal (checklist) and whether the patient is in remission (yes/no) will be collected.

**Follow-up phone visits and blood draw Maintenance Period = Visits Week
20, 28, 36, 44**

- Measurement of laboratory parameters
 - Hematology
- Phone interview: Phone interview with patient about adverse events (interview can be as well conducted in study center if feasible)

Follow-up visits Maintenance Period = Visits Week 24, 32, 40

Examinations

- Vital signs: blood pressure (mm Hg), heart rate (min-1), weight (kg)
 - Physical examination:
 - Head (including ENT and eyes)
 - Lymphatic system
 - Endocrine system
 - Lungs and respiratory tract
 - Peripheral vascular system
 - Cardiovascular system
 - Gastrointestinal tract, liver, spleen
 - Urogenital system
 - Nervous system
 - Muscular and skeletal system
 - Skin and connective tissue
 - Other
- Each: actual status: Present finding / normal condition / not examined
- Measurement of laboratory parameters
 - Hematology
 - Serum chemistry
 - Urine pregnancy test

Formal aspects:

- Return of study medication (empty vials)
- Calculation of Mayo score excluding the sigmoidoscopy score
- Compliance assessment (count of empty vials returned)
- Concomitant medication
- Adverse events
- Appointment for next visit

Final Visit Maintenance Period = Week 48 / premature withdrawal
Maintenance Period

Examinations

- Physical examination:
 - Head (including ENT and eyes)
 - Lymphatic system
 - Endocrine system
 - Lungs and respiratory tract
 - Peripheral vascular system
 - Cardiovascular system
 - Gastrointestinal tract, liver, spleen
 - Urogenital system
 - Nervous system
 - Muscular and skeletal system
 - Skin and connective tissue
 - Other

Each: actual status: Present finding / normal condition / not examined

- Sigmoidoscopy
- Vital signs: blood pressure (mm Hg), heart rate (min-1), weight (kg)
- Measurement of laboratory parameters
 - Hematology
 - Serum chemistry
 - Stool sample for calprotectin stool levels
 - Urine pregnancy test

Formal aspects:

- Return of study medication (empty vials)
- Calculation of Mayo score with the sigmoidoscopy score
- Compliance assessment (count of empty vials returned)
- Concomitant medication
- Adverse events

In case of **premature withdrawal** from the study (yes, no)

If yes, reason:

- Intolerance of medication
- Lack of efficacy (need for steroids e.g. increase of steroids or re-introduction of steroids after previously successful taper)
- Severe adverse event

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- Lack of patient's cooperation
- Inclusion criterion not fulfilled or exclusion criterion fulfilled coming to knowledge after recruitment
- Need for steroid therapy
- Other reason

Follow Up after premature withdrawal

If a subject withdraws before the completion of the study, follow up data will be collected one and three months after withdrawal. Information about which treatment options were started after withdrawal (checklist) and whether the patient is in remission (yes/no) will be collected.

Endoscopy and endoscopic assessment (week 0 and week 48/Premature Withdrawal Maintenance Period)

The endoscopy performed at week 0 should be a colonoscopy for patients that have a diagnosis of UC for ≥ 8 years and have not had a screening colonoscopy for ≥ 1 year. This colonoscopy would be considered as standard of care. For all other patients and all other visits, a sigmoidoscopy should be performed. Regardless of the type of endoscopy performed, only the 30 cm on retraction of the scope should be used for Mayo scoring purposes (range from 0 to 3 points). Photographs depicting examples of each of the 4 endoscopic subscores will be provided to sites for standardization purpose and the endoscopist should refer to these photos during the procedure to guide assignment of the endoscopy subscore. The endoscopy subscore at week 0 should be at least ≥ 2 . The endoscopies should be photo documented the pictures either digital or as print sent to the data management center.

Laboratory parameters

All laboratory analyses will be carried out in a central laboratory. All samples including C. diff toxin will be sent by overnight courier to this laboratory. Dry ice will be delivered to the participating sites if necessary.

Acute phase reactants (nonspecific inflammatory markers)

- C-reactive protein (CRP)

Hematology:

- Blood count (erythrocytes, hemoglobin, hematocrit, leukocytes, platelets and differential WBC)

Serum chemistry:

- Kidney function: serum urea and creatinine

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- Liver function (alanine aminotransferase {ALT}, aspartate aminotransferase {AST}, γ -glutamyl transferase {GGT}, alkaline phosphatase, bilirubin), albumin
- Other serum parameters (total protein, Na⁺, K⁺, Mg)
- Folate serum level at screening visit only

Hepatitis B and C screening

HBsAg, anti-HBs, anti-HBc, Hep-C AB

Stool sample for C. diff .toxin

Purpose of the DNA, plasma and serum repositories

The purpose of the repositories for DNA, serum and plasma is the evaluation of MTX pharmacogenetics and drug metabolite levels in the participating patients to evaluate criteria for MTX efficacy. MTX has a complex intracellular pathway and several genes have been associated with toxicity and response to MTX. Inter-individual variation in response and toxic side effects of UC patients to treatment with MTX may be influenced by inter-individual variation in candidate genes. We will also obtain DNA extracted from the buffy coat of blood samples drawn at inclusion into the study. Aliquots of serum and plasma will be collected from blood drawn at the same time. All specimens will be stored at -80°C at the central laboratory for the duration of the trial and after the successful completion of the study at the NIDDK Central repository indefinitely. All samples and data information will be de-identified.

5 RECORDING OF COMPLIANCE

Compliance will be assessed by checking the study medication (vials) returned at the follow-up visits and the final visit by the investigator.

During the Induction and Maintenance Period all returned vials will be counted by the investigator and the number will be documented in the electronic CRF.

6 ADVERSE EVENTS

Definitions

Adverse events (AEs)

Adverse events (AEs) will be recorded at each regular scheduled study visit or study phone contact in the patient record (source document) as well as on a specific AE form on the electronic CRF.

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An AE is any untoward medical occurrence in a patient or clinical investigation patient administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product, e.g.:

- any new diagnosis
- any symptom that requires medical clarification or leads to in-patient admission (surgery or accident)
- any suspected adverse drug reaction (ADR)
- any symptom that appears on the patient's medical records
- any event related in time with the application of the study medication and affecting the health of the patient (including laboratory value changes)

Serious Adverse Events (SAE)

A serious adverse event (experience) is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapacity

Non-serious adverse events are all AEs that do not fall into any of the above categories.

Unexpected Adverse Drug Reactions

An unexpected adverse drug reaction (ADR) is an adverse reaction, the nature or severity of which is not consistent with the applicable product information.

Expected Adverse Drug Reactions

Common ADRs caused by methotrexate include the following:

- anorexia, nausea, vomiting
- elevation of liver enzymes (AST, ALT , AP or GGT)
- stomatitis
- malaise
- fatigue
- abdominal discomfort

- chills
- fever
- dizziness
- diarrhea
- anemia
- thrombocytopenia
- leukopenia
- rash
- pruritus
- alopecia
- photosensitivity
- abortifacient, teratogenic

7 DOCUMENTATION AND REPORTING OF ADVERSE EVENTS

The patients will be instructed to contact the investigator, if any serious or unexpected AE occurs, so that appropriate action can be taken.

Moreover, the investigator must ask at each follow-up visit a generally worded question without searching for any special symptoms, e.g. “Has your state of health worsened since we last met?” If the answer to this question is “no”, no further questions will be asked. If the answer to the question is “yes”, the investigator will document the nature, time, severity, seriousness and duration as well as the causality of the AE. For each AE a specific AE documentation form will be provided to the investigators. In case of an AE, this should be completed by the physician as an initial report, and sent (via fax) to the principal investigator within 72 hours. The report must contain a detailed description of the symptoms observed and the concomitant treatment administered. Furthermore, the investigator must comment on a possible causative relationship between the AE and the trial medication. Each AE must be followed until it is resolved or can be explained satisfactory.

The following has to be documented for each AE:

- Nature of the event
- Time of onset: date, time
- Concomitant treatment: product (generic name), indication, dosage, dosage interval, presentation, mode of administration, administration regimen
- Duration of the AE

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- Severity
- Seriousness
- Causality
- Outcome

Severity

The severity is evaluated as follows:

1. Mild: - event/symptom does not interfere with normal daily activities
2. Moderate: - event/symptom interferes with normal daily activities
3. Severe: - event/symptom prevents normal daily activities

Causality

The relationship between an AE and the study medication is classified according to the WHO classification:

Certain

A clinical event, including laboratory test abnormality, is occurring in a plausible time relationship to drug administration, and which concurrent disease or other drugs or chemicals cannot explain. The response of the patient to withdrawal of the drug should be clinically plausible. The event must be definite pharmacologically or phenomenologically, using a satisfactory rechallenge procedure if necessary.

Probable/Likely

A clinical event, including laboratory test abnormality, with a reasonable time sequence to the administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal (de challenge). Rechallenge information is not required to fulfil this definition.

Possible

A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.

Unlikely

A clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations.

Conditional/Unclassified

A clinical event, including laboratory test abnormality, reported as an adverse reaction, about which more data are essential for a proper assessment or the additional data are under examination.

Not assessable / Unclassifiable

A report suggesting an adverse reaction, which cannot be judged because information is insufficient or contradictory, and which cannot be supplemented or verified.

As an alternative to the above-mentioned WHO assessments, the following assessment can be made:

Not related

There is sufficient information available to show that the etiology is unrelated to the study medication.

Measures at the onset of adverse events

Measures at the onset of adverse events are classified and described as follows:

- 1) None, i.e. the study medication was not changed
- 2) The dose of the study medication was reduced
- 3) The study medication was withdrawn and/or
- 4) Other measures (clear text)

The **course and outcome** of the adverse event will be commented on as follows:

- 1) Recovered without sequelae
- 2) Not yet recovered
- 3) Recovered with sequelae
- 4) Fatal

Documentation and Reporting of Serious Adverse Events

On enrolment in the study, the patients will be instructed to contact the investigator if a serious or unexpected AE occurs, so that appropriate measures can be taken.

Any SAE (including death, irrespective of the cause) occurring during or for up to 14 days after the end of the study must be reported without delay, i.e. within 24 hours, by telephone and by fax to the principal investigator of the study organization or his designee, irrespective of its relationship with the administration of the study medication (minimum information required: investigator's name/study center, patient number, patient initials, date of first dose, date of last dose, date of event, description of event, causality assessment, and countermeasures).

Address:	<u>Principal investigator</u>	Phone: 919-843-9071
Dep. of Internal Medicine	Hans Herfarth, MD, PhD	
130 Mason Farm Rd.		Fax: 919-843-6633
Chapel Hill, NC, 27599-7080		

A specific AE documentation form will be provided to the investigators (see appendix). In case of an SAE, this should be completed by the physician as an initial report, and sent (via fax) to the principal investigator. The report must contain a detailed description of the symptoms observed and the concomitant treatment administered. Furthermore, the investigator must comment on a possible causative relationship between the AE and the trial medication. Each SAE must be followed until it is resolved or can be explained satisfactorily.

The general procedure for the observation, collection and analysis of drug risks (regulatory affairs) in conformity with the appropriate national Drug Law shall apply without qualification.

In accordance with drug safety and national requirements, the study PI will inform the Data and Safety Monitoring Board (DSMB) of the study and will make sure that the involved persons will obtain adequate information. Also the local principal investigator will inform the local Ethics Committee.

The following instructions must be heeded:

In the case of an intolerable SAE, the patient must, at the decision of the investigator, be withdrawn from the clinical trial, and symptomatic treatment must be administered. The measures taken must be recorded on the electronic CRF.

In accordance with local legislation, the investigators will submit copies of the final SAE-report to the Regulatory Authorities concerned, if necessary.

8 ABNORMAL LABORATORY RESULTS

All laboratory values outside of the normal range will be repeated if judged appropriate by the investigator to ensure the validity of the abnormal result. The investigator will document all laboratory values on the relevant page of the electronic CRF and will assess the etiology of the clinically relevant abnormal laboratory values.

Clinically relevant abnormal laboratory values are only documented on the AE form of the electronic CRF, if the patient is discontinued, hospitalized, or, in the investigator's opinion, it should be considered as an AE.

Abnormal laboratory results caused by ulcerative colitis should not be reported on the AE form.

Monitoring of abnormal liver enzymes and complete blood count

- See appendix for summary of actions for abnormal lab values
- If the AST or ALT (LFT's) increase ≥ 2 times the upper limit of normal after the start of the study medication, the investigator has to repeat the measurements of LFTs after 2-3 weeks. If LFTs normalize the patient will continue with current dose of MTX, however, if LFTs remain elevated ≥ 2 times the upper limit at second draw, the dose of methotrexate should be reduced to 15 mg/week. Another check of LFT's should be performed after 4 weeks at the lower dose. If the LFT's normalize or remain stable, then further laboratory controls of the LFT's should be performed in the setting of the regular lab draws in the context of the regular study visits.
- If WBC below $3.0 \times 10^9/\text{th/L}$, the investigator should stop therapy with MTX and perform another blood draw in the setting of an unscheduled visit 14 days \pm 3 days later. If WBC at this unscheduled visit is $\geq 4.0 \times 10^9/\text{th/L}$ then patient may resume MTX therapy at the lower dose of 15 mg MTX/week. The next laboratory control should be then performed in the setting of a regular scheduled visit or at least 4 weeks \pm 2 weeks after the dose adaption of MTX in the maintenance. If WBC should drop again below $3.0 \times 10^9/\text{th/L}$ stop therapy with MTX.

Stop of study medication in case of following pathologic laboratory values:

- renal dysfunction (serum creatinine levels exceeding 1.5 mg/dL or $130 \mu\text{mol/L}$ or increase of 0.5 mg/dL or $43 \mu\text{mol/L}$ compared to baseline value)
- hepatic dysfunction as defined as an increase of AST or ALT ≥ 3 times upper limit of normal after the start of study medication.
- Drop of WBC below $3.0 \times 10^9/\text{th/L}$ despite dose reduction of MTX after the start of study medication.

9 WITHDRAWAL OR STOP OF STUDY DRUG CRITERIA

Criteria in individual cases

Any patient can withdraw from the study at any time without personal disadvantages and without having to give a reason. The time of withdrawal, the results available up to that time, and, if known, the reason for withdrawal must be documented on the electronic CRF. Of note, that patients not tolerating mesalamine (5-ASA) in the Maintenance Period should stop the drug, but will be continued in the study. The stop of the mesalamine) 5-ASA should be documented in the CRF.

The investigator can also discontinue the study after considering the risk-to-benefit ratio, if he/she no longer considers the further participation of the patient justifiable. The date of and the primary reason for the withdrawal as well as the observations available at the time of withdrawal are to be documented on the electronic CRF.

Reasons leading to the **withdrawal** of a patient can include the following (primary reason must be determined):

- inclusion criterion not fulfilled or exclusion criterion fulfilled; coming to knowledge after recruitment
- patient's request
- technical/logistical reasons (e.g. a change in place of residence, not referred by family physician, etc.)
- other reasons (noting reason)

Reasons leading to the **stop of study drug but follow-up according protocol** can include the following (primary reason must be determined):

- lack of efficacy of the study medication, e.g.
 - need for a prohibited concomitant medication (e.g. need for steroids in the maintenance period)
- intolerable adverse events, e.g.
 - renal dysfunction (serum creatinine levels exceeding 1.5 mg/dL or 130 µmol/L or increase of 0.5 mg/dL or 43 µmol/L compared to baseline value)
 - hepatic dysfunction as defined as an increase of AST or ALT ≥ 3 times upper limit of normal after the start of study medication.
 - Drop of WBC below 3.0×10^9 /L despite dose reduction of MTX to 15 mg/weekly after the start of study medication.
- lack of patient's cooperation, e.g.
 - patient's request

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- lack of compliance (fails to attend the follow-up visits as agreed)
- existing pregnancy or intended pregnancy* (females, men), lactation (females)

(*Methotrexate should not be used for at least 3 months before planned pregnancy for men and women and should not be used during pregnancy or breast feeding)

In all patients who finish the study prematurely, a withdrawal examination should be carried out within 2 weeks after the last application of the study medication. The withdrawal examination must be conducted as a final examination and documented in the electronic CRF. It is most important to calculate at least the final Mayo score for each patient. If possible, an endoscopy should be performed and the blood samples should be taken. The investigator will continue to observe patients withdrawn from the study because of intolerable AEs until the findings have been clarified. Also the investigator should follow the patients, in whom the study drug was stopped according to the regular scheduled study visits.

Treatment recommendations in case of flare of disease

In the event of a flare up in the Induction or Maintenance Period, the patient should be treated according standard of care and at the discretion of the investigator. The treatment may include the application of steroids, treatment of an underlying GI-infection or hospitalization for closer observation and intravenous hydration and/or intravenous or oral immunosuppressive therapies (e.g. steroids). Also a premature withdrawal visit should be conducted, but even after withdrawal of the study drug, the patient should be followed up until completion of the Induction Period or Maintenance Period, depending on in which Period of the trial the flare up occurred.

Criteria for the termination of the whole study

If serious safety concerns arise, the coordinating investigator can terminate or interrupt the study by agreement with the sponsor. If new information on the risk-to-benefit ratio of the drug or on the treatment methods used in the study is obtained in the meantime, the coordinating investigator reserves the right to interrupt or terminate the project by agreement with the sponsor. Premature termination is also possible if the coordinating investigator, or the investigators and the sponsor if patient recruitment is insufficient and cannot be expedited by appropriate measures. Criteria for early termination of the study related to results of interim analyses are discussed in the section “Statistical Considerations”.

10 MEDICATION PREPARATION AND DISTRIBUTION

Methotrexate

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The drug kits will be sent by the University of Pennsylvania Investigational Drug Service (IDS) directly to the patient unless due to local regulatory requirements the site would prefer to distribute the drug kits. In this case, IDS will send the drug kit to the site and the site will send the kit to the patient. All kits will have a unique number and will contain at least 1 month supply of medication or placebo and syringes for administration. When a patient qualifies for randomization, the IDS will implement the randomization according to the Group the patient is in. Sites will be notified by fax and email regarding the date that the blinded study medication pack was shipped. The form that identifies the treatment assignment code will be retained in the source documents. The coordinator will contact the patient to ensure they received the drug kit and to note the date the patient began study drug. In the case where the drug is shipped to the site, the site will distribute the treatment pack to the patient and then contact the patient to be sure they received it and to note the date they began study drug. All drug kits will contain dosing instructions.

Once the screening visit of the patient is entered in the database, a screening number will be assigned and the patient will receive 1 vial of MTX 2 ml (25mg/ml) and 7 tablets folic acid (1 mg) and 2 tablets promethazine (25 mg) by mail delivered to his home address from the IDS. The patient should bring the MTX vial and the folic acid tablets to the next visit at week 0. Depending on the local regulatory requirements the drug may be as well delivered to the investigational site. Should the patient not qualify for the inclusion into the trial at week 0, the investigational site should collect and discard the single MTX vial and the folic acid tablets them.

Each patient will be trained at week 0 in the Induction Period in administration of the study medication by a nurse using sodium chloride at the participating study sites. An instruction page will be provided and the patients will be observed doing the first subcutaneous administration themselves. Patients will be instructed to administer the medication subcutaneously in either the abdomen or thigh; whichever is easier for the patient. The first administration of MTX will take place at week 0 (visit 1) at the participating center. After the first injection of MTX the patient should also start to take folic acid tablets 1 mg tablets once daily.

After the first injection is applied at week 0 of the Induction Period the patient will receive, in regular time intervals, study drug kits containing (1)unblinded 2 ml vials MTX (concentration 25 mg/ml); (2) 1 ml syringes (U100 27g ½ inch syringes); (3) extra needles; (4) alcohol swabs; and (5) containers for sharps disposal. The disposal boxes can close to prevent sharps from falling out. The boxes will be returned to the study site for disposal during study visits. Also additional folic acid tablets for the remaining 16 weeks in the Induction Period will be sent to the patient. The patient will inject each week on the same day 1 ml (1 syringe) of MTX subcutaneously. The 1 ml syringes will be an extra caution to prevent MTX overdosing.

In the Maintenance Period the patient will receive in a double-blinded fashion either one 2ml vial MTX (concentration 25 mg/ml) or one 2 ml vial placebo. Due to the yellow colour of MTX, placebo will be prepared and packaged into matching 2 ml vials for subcutaneous injection using vial 2 of Infuvit® Adult (multiple vitamins for infusion), which is a FDA approved formulation for multiple vitamins for infusion. The vitamins will be diluted with sodium chloride to match the yellow color of the MTX and have maximal the following

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concentrations (values given for undiluted solution): Biotin 12 microgram/ ml, folic acid 120 microgram/ml and vitamin B12 1 microgram/ml. In addition both vials will be covered with an opaque foil-backed label with strong adhesive.

Folic acid tablets

Patients will be supplied with seven 1 mg folic acid tablets after the screening visit and once the patients are dosed with the first injection of MTX additionally folic acid tablets for the remaining 16 weeks will be sent to the patients. Patients entering the Maintenance Period will be supplied with 1 mg folic acid tablets for 48 weeks.

Promethazine

2 promethazine 25 mg tablets will be provided by the investigational drug service (IDS) directly to the patient after the screening visit. If the patient experiences nausea after the first injection the patient should be advised to take 1 promethazine tablet. In case of nausea the patient should also be advised to call the local coordinator for further supply of promethazine, which could be ordered by weborder from the investigational drug services (IDS). The IDS will then supply the patient with one promethazine tablet for each weekly MTX injection.

Ondansetron

If promethazine is not sufficient to suppress the MTX induced nausea, the patient should call the local coordinator. The coordinator should query the subject about a personal or family history (first degree relatives only) of long QT syndrome. If a history is present, ondansetron will not be ordered and the local investigator may prescribe an alternate anti-nausea therapy. Additionally, the investigator should review the subject's most recent laboratory results. If significant electrolyte abnormalities are present on the last lab draw (potassium < 2.8 mEq/L, magnesium <1.0 mEq/L), the patient should then undergo a portable EKG at the investigator site to exclude a long QT-syndrome. The QTc interval should not be longer than 440 msec. Once the patient has been excluded to have significant electrolyte abnormalities (potassium, magnesium) or a history or newly diagnosed long QT-syndrome, the investigator can directly order ondansetron 4mg tablets from the IDS for the patient. The IDS will then supply the patient with one ondansetron tablet for each weekly MTX injection.

The above precautions are necessary; since on 9/15/2011 the FDA issued a warning that ondansetron may increase the risk of developing abnormal changes in the electrical activity of the heart, which can result in a potentially fatal abnormal heart rhythm. Patients at particular risk include those with underlying heart conditions, such as congenital long QT-syndrome and those who are predisposed to low levels of potassium and magnesium in the blood.

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Mesalamine (Asacol HD)

16 weeks supply of mesalamine tablets will be provided by the investigational drug service (IDS) directly to the patient once the patient has been randomized in the Maintenance Period and after 15 weeks in the Maintenance Period. If subjects have a known intolerance of 5-ASA, they will not receive Asacol HD from the IDS during the Maintenance Period.

11 DATA SAFETY MONITORING

On a monthly basis, the DCC will issue standard safety reports (enumerated by event and category, both by total and by type) as well as a cumulative report of all Adverse Events (AEs) to include Event Code, Dates of Onset/Resolution, Grade, and Outcome to the an independent safety monitor. In addition, these standard safety reports will be presented to the DSMB at each conference. The DSMB will review these data and make appropriate safety decisions based on these events. Participants will be monitored for potential AEs, including signs or symptoms related or unrelated to the condition under study, any clinically significant laboratory abnormality, or any abnormality detected during physical examination.

12 RANDOMIZATION, DATA MANAGEMENT AND DATA MONITORING

The randomization of the patients and the processing and analysis of the data will be carried out by the Biostatistics core of the Center for Gastrointestinal Biology and Disease at UNC Chapel Hill.

Study data for this study will be collected and stored using electronic records. Data captured will be entered in real time at each clinical site using web forms developed to replicate paper case report forms. All data will be created, modified, maintained, archived, retrieved and distributed by a computer system. The use of electronic records will increase the speed of data collection and exchange. This will reduce the manpower necessary to perform double-data entry from paper forms and transcription error. In addition, electronic records permit economical storage of study data and ease of accessibility and analysis. Data management and data quality systems will be built into the system.

Data quality using electronic records will ensure that data are attributable, legible, contemporaneous and original.

The Data Management Center (DMC) at the CGIBD at the University of North Carolina will track the data collection, provide data security, control for confidentiality of study data, maintain computer backups to protect data until study closure and archive study data according to FDA requirements (21 CFR 11). Electronic signatures will be linked to each entry.

All computer systems and programs will be password protected, and all electronic communications of study and other confidential information will be encrypted. Personnel at the CGIBD have extensive training and experience using electronic data systems. Good computer security practice (restricting physical access to machines, prohibition of password sharing, and logging off computers after work hours or when away from the machine) will be required of all study personnel.

Standard Operating Procedures exist for users of the DMC. Only authorized persons are authorized for data entry and access. Data security systems require password protected identification codes for data entry and provide protection against data manipulation. The database is located on a server protected by firewalls. Access to the database server will not be allowed by users on computers outside of the firewall-protected zone. Virus protection software is installed on each study machine. System access to computer systems will be audited. Redundant backups and off-site backup storage will allow for quick restoration of data in the unlikely event that a hardware failure, disaster, or security breach should occur. Servers and backups will be located in a secured location with access limited to authorized personnel.

Data cleaning will include range and edit checks, cross form edit checks, query generating and tracking and periodic data status reports. Any data errors or inconsistencies detected after data entry will be automatically tracked, communicated and resolved using a web-based application. An audit trail of all data changes over the life of the study will be maintained. All study raw data, forms, documents, software programs, software applications and computer data files will be indexed and archived routinely. Strict version control of documents and software applications will be instituted. Retention of study documentation after study completion will conform to FDA and NIH requirements.

Standardized study management reports will be generated monthly during the recruitment phase of the study. These reports will be used to track study progress including patient enrollment, randomization, compliance, patient status changes, and study events. The data will be reported for each Study Center individually and summarized for the study as a whole. Every six months, a standardized report will also be generated for the DSMB meeting. This report will include additional information on clinical events and adverse events that is coded by blinded treatment group. Other than the study statistician and statistical analyst, no study personnel will see this report.

13 STATISTICAL CONSIDERATIONS

Sample Size Calculation

The primary aim is to test whether the relapse rate of patients randomized to MTX maintenance therapy following 16 weeks of successful MTX treatment is clinically superior to the rate of similar patients randomized to placebo maintenance. To test this, we will compare relapse-free survival rates in each treatment group over the 32 week follow-up period using the log-rank test for a difference in survivor functions²³⁻²⁵. We estimate rates of relapse-free survival in the placebo group will range from 38% to 42%. We also estimate

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that improvements in survival rates of 25% or greater are of clinical significance given the widespread availability and low cost of this intervention compared to alternatives. This is equivalent to 52-54% reduction in hazard over the expected range of placebo group relapse-free survival rates.

Since there are no known data of the clinical efficacy of MTX in prospective placebo controlled trials, we assumed a success rate (clinical remission and off steroids or clinical response and off steroids as defined by the partial Mayo score) of 55-70% with open label MTX and steroid taper after 16 weeks (see table A). This estimate is based on previous publications with open label administered MTX as described in the Background section of the protocol ^{14, 19-22}. We postulate an absolute difference of 25% between the MTX (65% maintenance of remission and off steroids until week 48) and placebo group (40% maintenance of remission and off steroids until week 48).

These assumptions were based on the following data:

- A 26% difference in successful maintenance was observed between MTX 15mg/week vs. placebo in the landmark study investigating the efficacy of MTX in maintaining remission in CD patients by Feagan et al. ⁹. In this study maintenance therapy with MTX resulted in 65 % remission and no steroids at week 40 compared to 39% in the placebo group.
- Previous trials in steroid dependent UC patients despite 5-ASA therapy demonstrated remission rates of 53% on azathioprine and 21% on 5-ASA after 6 months ²⁶ and in patients on long term azathioprine a 61% relapse rate after withdrawing this drug compared to 31% in patients continuing on azathioprine ²⁷.

The number of patients randomized depends on the proportion of those enrolled in the induction phase, who achieve MTX-induced remission or response and of the proportion who agree to continue with the 32 week blinded maintenance phase. Table A shows the numbers of patients (both arms) who would be randomized based on estimated induction phase remission rates of 55%-70%, under scenarios of 180-220 patients being enrolled into the induction phase, allowing for a loss of 5% of patients in remission or responding to MTX, who do not continue with the maintenance phase. For instance, if 200 patients are enrolled in induction phase, and remission is 65%, and 5% do not continue, 124 will be assigned to MTX or placebo and available for analysis.

Table A. Number of patients randomized to maintenance trial under varying remission rates, for different numbers screened and enrolled into induction phase.

Number needed to screen			Induction Period			
Screen failure rates		Number	Remission/response week 16			
30%	25%	Enrolled	0.55	0.60	0.65	0.70

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257	240	180	94	103	111	120
286	267	200	105	114	124	133
314	293	220	115	125	136	146

The middle range of response or remission rates (60-65%) leads to randomized study population sizes of 103-136 patients if 180-220 are enrolled in induction phase. We will monitor remission rates during the induction phase to determine the number needed to enrol to reach our required sample size for the randomized maintenance phase. If remission rates are higher, fewer patients will be needed overall; if lower, we will continue to enrol until we reach our target study size.

We calculated statistical power for log-rank tests of differences in survival using Stata version 11 (Austin TX), based on Schoenfeld et al.²⁵. The power available to detect a 25% improvement in survival (40% on placebo versus 65% on MTX) is equal to 75% if 103 patients are randomized to MTX or placebo with 50/50 allocation ratio, and is equal to 86% if 136 patients are randomized. This power calculations are based on the assumption that time to even is exponentially distributed and take into account an interim analysis for efficacy using O'Brien-Fleming boundary.

136 patients randomized equally to MTX or placebo ranges between 75% and 86%.

Using a two-sided type 1 error rate of 0.05, and assuming a screen failure rate of 30% (which is based on the recent trials using infliximab and abatacept for the same patient group), we calculated for our current protocol that we would need to screen 314 patients to be able to enrol 220 patients in the Induction Period to be able to randomize a total of at least 119 patients, which will give the study a statistical power of at least 80% percent. This will provide sufficient power to detect a clinically significant 25% improvement in survival based on the range of expected survival rates in the placebo group. Depending on actual induction remission rates, we will need to enrol between 180-220 patients in the open label induction phase, which implies screening between 240-324 patients at screening failure rates of 25-30%.

Efficacy analysis population

The efficacy analyses will be performed using the intention-to treat (ITT) principle. That is, all patients receiving at least one injection of study medication after randomization at week 16.

The statistical analyses of the primary and secondary endpoints will be performed with the statistical support of Joseph Galanko, PhD, who is the biostatistician at the Biostatistics Core of the Center for Gastrointestinal Biology and Disease at the University of North Carolina and Christopher Martin, MSPH, who is the director of the Biostatistics Core of the Center

for Gastrointestinal Biology and Disease at the University of North Carolina. SAS software (SAS Institute, Cary NC) will be used to perform all analyses.

Statistical analyses

The primary aim is to test effectiveness of MTX in maintaining clinical remission over 32 weeks of therapy compared to placebo. Analyses of this aim will be conducted at two time points: (1) final analysis at the conclusion of the trial and (2) one interim analysis when about half of the patients completed follow-up in the maintenance phase. The interim analysis is planned to allow the DSMB to determine whether treatments are so convincingly different that continuation of the trial would be unethical, and also whether side effects of treatment are too severe to warrant continued therapy given the potential risk: benefit ratio. Details and methods of these final and interim analyses are described below.

In addition, we plan to conduct a single test of open label induction phase response rates after the first 75 patients have completed this phase of the trial. Accrual of sufficient numbers of patients into the randomized maintenance trial depends on the effectiveness of MTX in achieving remission during the 16 week open label induction phase. Therefore, after the first 75 patients have completed the induction phase, we will assess whether observed response rates are sufficient to meet goals for accrual to randomization in a timely manner, and also to determine whether inclusion/exclusion criteria should be modified to target subgroups of patients most likely to benefit from induction treatment.

Analysis of induction phase response rates

Each patient will be categorized as a success or failure with respect to response at 16 weeks. Response at week 16 is defined as either clinical remission or clinical response: (1). Clinical remission is a partial Mayo score of no more than 2 points with no individual subscore exceeding 1 point, *and* no use of steroids at week 16; (2) clinical response, defined by a reduction of the partial Mayo score ≥ 2 points and at least 25% with an accompanying decrease in the subscore for rectal bleeding of at least 1 point or an absolute subscore for rectal bleeding of 0 or 1 and has a clinical Mayo score of ≤ 5 , *and* no use of steroids at week 16 and a partial Mayo score ≤ 5 .

Because only patients who achieve response during the induction phase are randomized into the maintenance therapy, successful accrual into the randomized maintenance trial depends on the minimal proportion achieving response during induction. Based on expected numbers of patients available for enrollment into induction phase, and the number required to meet minimum power in the randomized trial, we have determined that induction rates need to a minimum of 30% or more to ensure sufficient accrual into the trial during the time allowed.

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Patients who discontinue treatment prior to 16 weeks for any reason will be categorized as failures. As soon as 75 patients have completed the 16-week induction phase, we will compute the point estimate and Clopper-Pearson exact 95% confidence interval of the proportion of successful induction therapy.

Patients who discontinue treatment prior to 16 weeks for any reason will be categorized as failures. To achieve our enrollment goals in the planned time frame, we estimate that the rate of remission/response during induction should be at least 30%. Therefore, we will assess the success rate as soon as the first 75 patients have completed the 16-week induction phase. If the proportion achieving remission/response does not exceed 30% (23 or more patients achieving this outcome), the sponsor and the DSMB will review the data and decide if the trial should be stopped or modified and continued.

If the proportion achieving remission/response exceeds 30%, we will continue enrollment as planned. However, we will utilize this assessment of remission/response rates to investigate possible revision of inclusion criteria to improve accrual. Specifically, we will compare remission/response rates across strata of patients defined by pre-enrollment therapy: steroid-dependent only, azathioprine/6-MP failure, azathioprine/6-MP intolerance, anti-TNF failure, or anti-TNF loss of response. If one or more of these subgroups has significantly higher remission/response rates, we will explore whether revision of inclusion criteria to preferentially recruit patients most likely to respond to induction therapy will enhance accrual rates into the randomized phase.

We recognize that there is a degree of uncertainty around the remission/response rate yet our decision to stop the trial would be based on a point estimate of response of at least 30%. With 75 evaluable subjects, the confidence interval around the 30% response rate would be 20% to 42%. Thus, with 75 subjects, we will have relatively precise estimates of response/remission rates. Even were the response/remission rate as high as 42%, the medication would be viewed as relatively modestly effective. We would reserve the right to discuss this stopping rule with the DSMB if the proportion of the responders who have achieved remission is high, understanding that greater value may be placed on remission than response by patients and treating physicians.

Primary analyses

The primary aim of the trial is to test whether primary and secondary outcomes after 32 weeks of maintenance therapy differ between patients randomized to MTX versus those randomized to placebo. As described previously, one interim analysis of primary outcomes is planned for DSMB review during the course of the trial. Both the primary and interim analyses will be based on intent-to-treat population. That is, patients will be classified according to the treatment arm to which they were randomized, without respect to compliance, drop out, or other drugs taken. We also plan secondary per-protocol analyses after completion of primary intent-to-treat analyses.

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The total period of enrollment is 48 weeks, consisting of 16 weeks open label induction, followed by 32 weeks of randomized maintenance therapy for patients who achieve remission during the induction phase. The follow-up time for planned primary comparisons of treatment-specific treatment survivor functions is restricted to the 32-week maintenance phase. The colonoscopy exam at the end of follow-up occurs at week 48 of enrollment, which is week 32 of follow-up. Thus, we refer to outcome assessment below as occurring at week 32.

Definition of primary and secondary outcomes

The primary outcome is relapse-free survival, comprised of three components, all of which must be met to be categorized as relapse-free: total week 32 Mayo score not exceeding 2 points, with all individual subscores not exceeding 1 point and relapse free survival defined by a numerical stable Mayo score throughout 32 weeks of maintenance therapy without increase of 3 or more points in the partial Mayo clinic score (excluding sigmoidoscopy) compared to the partial Mayo score of the individual patient at randomization at week 16 (2) and no steroid use or other immunosuppressive medication (anti-TNF agents, thiopurines, cyclosporine, tacrolimus) to control disease activity throughout the 32 week maintenance period. For most patients, time-to-failure or censoring will occur at the final visit (32 weeks) when the Mayo score is determined, however for patients who relapse due to initiation of steroid treatment before 32 weeks, time-to-failure will be the week in which steroid therapy was started.

Two secondary outcomes are also defined: (1) mucosal healing, defined as an absolute subscore for endoscopy no more than 1 at week 32, and (2) relapse of disease, defined as an increase of 3 or more points in the partial Mayo clinic score (excluding sigmoidoscopy) with an absolute clinical Mayo score ≥ 4 , or re-treatment with steroids during maintenance. Any patients for whom outcomes cannot be assessed for any reason will be classified as failures (relapses) with the event time assigned as the date that the patient was withdrawn from the study.

Patient characteristics of interest

For assessment of randomization success, and for assessment of possible confounding of treatment effects with respect to primary and secondary outcomes, we will use the following list of patient factors: age, sex, previous medication use (5-ASA only, azathioprine/-MP, anti-TNF agents), extent of disease (proctitis, left-sided disease, or pan colitis) steroid dependent disease at the time of enrolment, entry Mayo disease activity score and smoking status.

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Analysis plan

Descriptive Statistics

We will also compute the treatment-arm-specific proportions and exact 95% confidence intervals of patients achieving each primary and secondary outcome of interest.

We will describe and summarize the bivariate distributions of patient characteristics (age, sex, duration of disease, previous therapies, smoking status, disease extent, disease severity) within treatment arms. We will compute medians, interquartile ranges, means and standard errors of continuous variables. Categorical variables will be summarized using proportions in each level. We will compare distributions across treatment arms as follows: (1) for categorical variables, Fisher's exact chi-square tests of association in 2-by-X tables, or (2) for continuous variables, either Student's t-tests of differences in means (for normally distributed variables), or Wilcoxon rank-sum tests for non-normally distributed variables. Because any imbalance in the two randomized groups is by definition a chance occurrence, these descriptive analyses will be used to highlight potential areas of substantial unbalance between the study arms and to inform adjusted analyses of treatment effect.

Analysis of primary and secondary outcomes

All statistical tests for the primary and secondary outcomes will be 2-sided using $\alpha=0.05$, except as described below with regard to the pre-specified interim analyses of the primary outcome for the DSMB.

The primary aim is to test whether the relapse-free remission in patients randomized to MTX maintenance therapy following MTX-induced remission is superior to that in similar patients randomized to placebo maintenance. This primary outcome will be determined for each patient, along with the time-to-failure or, for patients who do not relapse, time-to-censoring of 32 weeks. These data will be used to compute survivor functions within treatment groups. We will then compare treatment-group-specific survivor functions using the non-parametric logrank test. This tests the null hypothesis

$$H_0 : S_1(t) = S_2(t) \text{ for all } t \leq 32$$

where $S_j(t)$ is the survival function in group j at time t . For this test, we will use $\alpha=.05$ to account for the interim analysis (see below).

We fully expect randomization to result in balanced distribution of potential confounders across treatment groups. Further, as randomization will be by permuted blocks within site, we also do not expect confounding by study site. We will nonetheless assess possible confounding by any variables found to differ across treatment groups, and, also by site if treatment distribution differs across sites.

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We will also assess heterogeneity of effect by covariates of interest, and by site, using Mantel-Haenzel methods to compute and compare crude and summary incidence rate ratios, with accompanying tests for heterogeneity. If any heterogeneity is observed, we will report stratum-specific treatment effects.

We will repeat these analyses for the secondary outcomes.

Exploratory analyses of factors associated with remission at week 32 of Maintenance Period

If a treatment effect on relapse-free survival is observed, we will also conduct exploratory analyses of patient characteristics predicting remission versus failure among MTX-treated patients. We will use bivariate methods to assess associations of each patient factor of interest (described above) with relapse-free remission at week 32, followed by multivariable logistic regression modeling having relapse-free remission as the dependent variable. To assess relationships of each factor to this outcome while adjusting for other potential confounders, we will use a 10% change-in-estimate criterion to assess whether individual covariates should be retained.

Exploratory analyses of factors associated with successful induction therapy

We will conduct exploratory analyses of patient factors predicting achievement of remission and clinical response following 16 week of open label MTX treatment (Induction Period). The methods used will parallel those just described used for exploratory analyses of factors associated with relapse-free remission at week 32 of the Maintenance Period, except the outcomes of interest will be remission and clinical response at 16 weeks induction therapy.

DSMB interim analyses and early stopping rules for interim analyses

Interim analyses of treatment effect will be conducted when 50% of patients will have completed the Maintenance Period of the study (which will be approx. at the end of year 2). Results of the interim analysis will only be provided only to the Data Safety Monitoring Board, not to the investigators. This interim analysis will use the methods described above using logrank tests to detect treatment group differences in survivor functions with respect to the primary outcome, relapse-free maintenance of remission. The absolute and relative difference in survivor functions, if any, will also be summarized. Accompanying these results will be summaries of the distributions of covariates of interest by treatment group, as explained in Descriptive Analyses. In addition, summaries of adverse events will be calculated by treatment group with accompanying chi-square tests to indicate statistically different incidence of adverse events by treatment group.

By definition, interim analyses require multiple analyses of the data. To avoid inflating the overall type I error rate for the principal analysis of efficacy, we will use the O'Brien-Fleming method of adjusting type I error rate for planned interim analyses. This design

utilizes very low type I error rates early in the trial, to preserve a level at or near the traditional 0.05 for the final analysis. In addition, an inflating type I error rate is used for sequential interim analyses, increasing the probability of stopping the trial in later analyses when more data are available. As this trial accrues patients for over three years, there will be a total of two analyses (one interim analysis and one final analysis). The suggested stopping boundaries for the analysis of primary treatment effect are shown in table 1.

Table 1. Proposed stopping boundaries		
Analysis	Z	P
1	2.800	.0050
2	1.977	.0480

Since the DSMB might not meet when exactly half of the patients completed the study, it might be preferable to use the error spending function $f(t) = \min\{2 - 2\Phi(\Phi^{-1}(0.025/\phi(t)))\sqrt{t}, 0.05\}$, where Φ is the cumulative distribution function of standard normal and t is the information fraction observed in the trial at the time of interim analysis.

Any decision to stop the trial early due reasons of efficacy or safety will be made solely by the Data Safety Monitoring Board; these a priori stopping boundaries are merely intended to serve as a guideline to those decisions.

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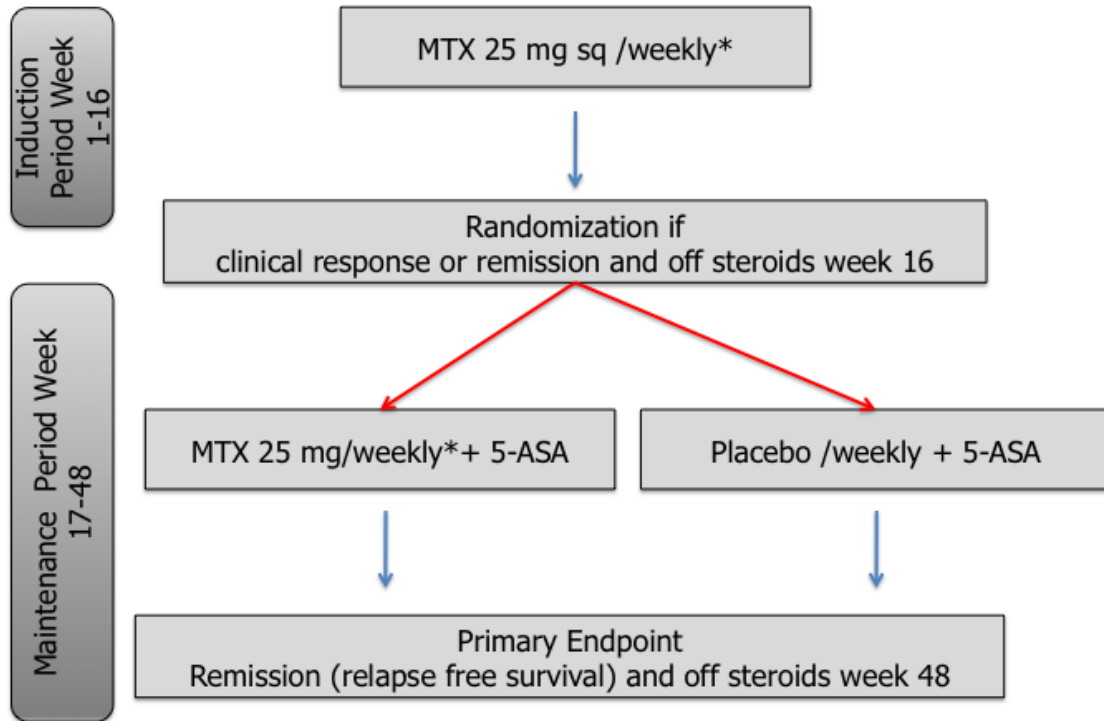
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15 APPENDIX

Study Flow Chart



* Dosis reduction to 15 mg sq/weekly in case of MTX side effects

Timeline study visits

[illegible]

AE, adverse event; CM, concomitant medication

* or 15 mg/weekly in case of dose reduction due to MTX associated side effects.

^a Diary entries from the 3 days preceding the study visit will be used to calculate the Mayo score

^b CBC, AST, ALT, GGT, alkaline phosphatase, bilirubin, albumin, total protein, creatinine, CRP, serum folate, magnesium, sodium, potassium, and hepatitis (screening visit only)

^c additional DNA sample for DNA, serum and plasma library. At week 16, plasma and serum sample only.

^d Stool sample for C. diff. at screening (screening visit) and stool samples for calprotectin (central laboratory) at screening, Week 16 and Week 48.

^e laboratory evaluation not necessary if inclusion in a time period of 14 days after screening visit

^f if a chest x-ray was obtained within 1 year before screening, there is no need for a new chest x-ray, but the results and the date of this x-ray should be recorded in the CRF

^g serum beta-HCG send to central laboratory

^h CBC only

Mayo Score

MAYO SCORING FOR ASSESSMENT OF ULCERATIVE COLITIS ACTIVITY

Stool frequency*

0 = Normal no. stools for this for this patient

1 = 1-2 stools more than normal

2 = 3-4 stools more than normal

3 = 5 or more stools more than normal

* Each patient served as his or her own control to establish the degree of abnormality of the stool frequency.

Rectal bleeding

0 = No blood seen

1 = Streaks of blood with stool less than half the time

2 = Obvious blood with stool most of the time

3 = Blood alone passed

Findings of flexible proctosigmoidoscopy

0 = Normal or inactive disease

1 = Mild disease (erythema, decreased vascular pattern, mild friability)

2 = Moderate disease (marked erythema, absent vascular pattern, friability, erosions)

3 = Severe disease (spontaneous bleeding, ulceration)

Physician's global assessment**

0 = Normal (there are no symptoms of colitis, the patient feels well, and the flexible proctosigmoidoscopy score is 0) (stool frequency = 0, rectal bleeding = 0, patients functional assessment = 0, flexible proctosigmoidoscopy findings = 0)

1 = Mild disease (mild symptoms and proctoscopic findings that were mildly abnormal) (the subscores should be mostly 1's: stool frequency = 0 or 1; rectal bleeding = 0 or 1; patients functional assessment = 0 or 1; sigmoidoscopy findings = 0 or 1)

2 = Moderate disease (more serious abnormalities and proctosigmoidoscopic and symptom scores of 1 to 2) (the subscores should be mostly 2's: stool frequency = 1 or 2; rectal bleeding = 1 or 2; patients functional assessment = 1 or 2; sigmoidoscopy findings = 1 or 2)

3 = Severe disease (the proctosigmoidoscopic and symptom scores are 2 to 3 and the patient probably requires corticosteroid therapy and possibly hospitalization) (the subscores should be mostly 3's: stool frequency = 2 or 3; rectal bleeding = 2 or 3; patients functional assessment = 2 or 3; sigmoidoscopy findings = 2 or 3)

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** The physician's global assessment acknowledged the three other criteria, the patient's daily record of abdominal discomfort and general sense of well-being, and other observations, such as physical findings and the patient's performance status.

Patient's functional assessment (this variable is not included in the 12 point index calculation but is considered as a measure of general sense of well-being when determining the physician's global assessment score)

0 = Generally well

1 = Fair

2 = Poor

3 = Terrible

1

Schroeder KW, Tremaine WJ, Ilstrup DM. Coated Oral 5-aminosalicylic acid therapy for mildly to moderate active ulcerative colitis. *N Engl J Med* 1987; 317: 1625-1629.

Subject Diary for Ulcerative Colitis														
Target Visit Date: _____														
Instructions: Begin recording in your diary each night, beginning three nights prior to your office visit. Your should record before going to bed and answer should describe the preceding 24 hours. The scores for abdominal pain and general well-being should reflect the average pain or well-being for that day. If you miss a day and you cannot recall all the information, complete the date and the information you do recall. An extra row is provided in case corrections need to be made														
Date	Number of bowel movements <u>greater</u> than normal					Blood in Stool	Abdominal Pain /Cramps	General Well Being				Did you take any medication for diarrhea?		
	0 = Normal					0 = No blood seen	0 = None	0 = Generally well				Yes/No		
	1 = One or Two					1 = Streaks of blood in stool or obvious blood in stool in < 50% of bowel movements	1 = Mild - aware but tolerable	1 = Fair						
	2 = Three or Four					2 = Obvious blood in stool in ≥ 50% of bowel movements	2 = Moderate – interferes with usual activity	2 = Poor						
	3 = Five or More					3 = Blood alone passed in any bowel movement	3 = Severe - incapacitating	3 = Terrible						
	0	1	2	3	0	1	2	3	0	1	2	3	Yes	No
	0	1	2	3	0	1	2	3	0	1	2	3	Yes	No
	0	1	2	3	0	1	2	3	0	1	2	3	Yes	No
	0	1	2	3	0	1	2	3	0	1	2	3	Yes	No

Clinical Adverse Event Reporting Form

Randomized, double blind, prospective trial investigating the efficacy of Methotrexate in induction and maintenance of steroid free remission in ulcerative colitis (MEthotrexate Response In Treatment of UC - Merit-UC)

Event ID: _____ Study Center: _____ Study ID: _____

DOB: ____/____/____ Gender: _____ Race: _____
 mm dd yyyy

Name of MD Reporting Event?	_____
Date of Medical Review	____/____/____
	mm dd yyyy
Is this a Serious Adverse Event (SAE)?	<input type="checkbox"/> Yes <input type="checkbox"/> No
Indicate all classifications(s) applicable	<input type="checkbox"/> Death <input type="checkbox"/> Life threatening <input type="checkbox"/> Disability <input type="checkbox"/> Congenital anomaly/birth defect <input type="checkbox"/> Hospitalization - Initial <input type="checkbox"/> Hospitalization - Prolongation <input type="checkbox"/> Other
Specify Other	
Relationship/Causality (likelihood that study agent <u>caused</u> the event)	<input type="checkbox"/> Not related <input type="checkbox"/> Probably not related <input type="checkbox"/> Possibly related <input type="checkbox"/> Related

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MD Narrative:	
Measures at the onset of adverse event	<input type="checkbox"/> None <input type="checkbox"/> Reduction of the dose of study medication <input type="checkbox"/> Withdrawal of the study medication <input type="checkbox"/> Other
Specify Other:	
Outcome of event (sequelae are defined as conditions following and resulting from event)	<input type="checkbox"/> Recovered without sequelae <input type="checkbox"/> Recovered with sequelae <input type="checkbox"/> Not yet recovered <input type="checkbox"/> Died
Signature of Study Center MD	_____
Date Form Completed	____ / ____ / ____ mm dd yyyy

Summary of Abnormal Lab Values and Required Actions

	Abnormal value	Action after first lab	Action after FU lab	Action after
WBC	< 3 x 10 ⁹ L	<ol style="list-style-type: none"> 1. Call patient and advise to stop MTX. 2. Arrange for FU lab in 14 days (+/-3 days) 3. Send AE report 4. Continue study visits 	>4 x 10⁹ L: <ol style="list-style-type: none"> 1. Call patient and advise to resume MTX at 15mg/wk. 2. Continue study visits 	<ol style="list-style-type: none"> 1. If value ever advise patient 2. Send AE r 3. Continue s
			<3 x 10⁹ L: <ol style="list-style-type: none"> 1. Call patient and advise to discontinue MTX 2. Continue study visits 2. Send AE report 	
ALT AST	≥ 2X upper limit	<ol style="list-style-type: none"> 1. Call patient and arrange for FU lab in 2 weeks 2. Continue with current dose of MTX 3. Send AE report 4. Continue study visits 	ALT normalized: <ol style="list-style-type: none"> 1. Continue with current dose of MTX 2. Continue study visits 	ALT > 2x up <ol style="list-style-type: none"> 1. Call patie MTX 2. Send AE r 3. Continue s
			ALT > 2x upper limit <ol style="list-style-type: none"> 1. Call patient and advise to reduce MTX to 15mg/wk. 2. Recheck ALT in 4 weeks 3. Send AE report 4. Continue study visits 	
Creatinine	>1.5 mg/dL	<ol style="list-style-type: none"> 1. Call patient and advise to stop MTX. 2. Arrange for FU lab in 14 days (+/-3 days) 3. Send AE report 4. Continue study visits 	Creatinine in normal range <ol style="list-style-type: none"> 1. Call patient and advise to resume MTX at 15mg/wk. 2. Continue study visits Creatinine >1.5 mg/dl <ol style="list-style-type: none"> 1. Call patient and advise to discontinue MTX 2. Send AE report 3. Continue study visits 	